MICROBIOLOGY REPORT ON SUDBURY ENVIRONMENTAL STUDY

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1974 MICROBIOLOGY REPORT ON SUDBURY ENVIRONMENTAL STUDY;

INFLUENCE OF RECLAMATION EXPERIMENTS ON THE MICROBIAL ECOLOGY OF SELECTED LAKES IN THE SUDBURY, ONTARIO DISTRICT

SECTION 1: Quantitative Evaluation of the Microbial Populations of the Reclamation Lakes After Treatment.

SECTION 2: Experiments on the Microbial Degradation of Natural Organic Substrates and Heterotrophic Productivity in Selected Lakes.

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SUMMARY-SECTION I

The purpose of the 1974 microbiological study of the Reclamation Lakes was to continue the evaluation of lime treatment on certain microbial populations. Additional external control lakes were introduced.

The heterotrophic bacterial populations in the treated lakes (Middle, Lohi) were comparable to those found in the external eutrophic controls (Minnow, Maclean), and significantly higher than in the untreated controls (Hannah, Clearwater).

Aciduric bacterial populations were higher in the untreated lakes and in the oligotrophic external control lake (Harp).

No significant differences were observed for sediment heterotrophic bacterial populations between the treated and untreated lakes. Higher levels of sulphate reducing bacteria were found in Middle and Lohi Lakes, and in the eutrophic controls.

Higher algal populations were observed in the surface water of the treated lakes than in the untreated lakes. Yeast levels were low in the water column of all lakes sampled.

SECTION I: N.E. CROLL and F.R. THOMPSON

Quantitative evaluation of the microbial populations of the Reclamation Lakes after treatment.

I.1 Introduction:

The accumulation of various airborne smelter emissions since the 1900's has resulted in the disruption of the ecosystem in the Sudbury area. McGovern and Balsillie (1974) described adverse effects on the vegetation near Sudbury, and Beamish (1974) showed fishery declines in the Killarney area. Similar problems have occurred in Sweden (Tyler, 1972; Almer, 1974; Grahn et al, 1974), where acidification and heavy metals have disrupted normal productivity in almost 1,000 lakes (Almer, 1974).

Until the International Nickel Company's (INCO) large (1250') stack was put into operation, any effects were relatively localized (Gorham and Gordon; 1960, 1963). In time, the same effects may be observed further afield as sulphurous contaminants are transported over larger areas. Airborne sulphur dioxide (SO_2) reacts with oxygen (O_2) and water (H_2O) in the presence of light to produce sulphurous (H_2SO_3) and sulphuric (H_2SO_4) acid, resulting in "acid rain" (Likens & Borman, 1974; Kramer, 1973). As a result, the pH levels in some poorly buffered lakes were reduced, primary producer populations declined, and productive fisheries were lost.

The combined effect of low pH plus high heavy metal concentrations (eg. >1000 ppb Nickel) in some lakes has been

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sufficient to further reduce productivity. These oligotrophic Precambrian Shield lakes had inherently low productivity due to naturally low nutrient levels, but the additional stress of acid rain was often sufficient to practically eliminate microbial productivity. The magnitude of disruption is a function of geophysical and geochemical characteristics (inherent buffering capacity), morphometry and environmental influences.

Early in 1973, four lakes (Lohi, Clearwater, Hannah and Middle) within 12 km of Sudbury were selected for study. Background data was collected prior to lime treatment in the fall of that year. Immediate positive effects were pH neutralization and increased heterotrophic bacterial populations. A more complete discussion of treatment and liming effects can be found in the M.O.E. Microbiology Report on the Sudbury Environmental Study, 1973.

Because of the 1973 results, the experimental approach for the 1974 microbiological sampling season was altered. The primary objectives were:

1) Enumeration of the heterotrophic bacterial populations such that control and treated lakes could be statistically evaluated;

2) Evaluation of aerobic and anaerobic bacterial sediment populations to trace any changes occurring as a result of liming;

3) Use of external area controls for comparison with the Reclamation Lake group;

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4) Evaluation of selected autotrophic populations in both the water and sediment;

5) Evaluation of blue-green algae populations which are involved in nitrogen (N_2) fixation;

6) Development of the adenosine triphosphate (ATP) method for the determination of total biomass;

7) Study of the effects of selected substrate additions by in situ incubations.

Description of Study Area:

The external control lakes selected for study were Harp (oligotrophic), Maclean and Minnow (eutrophic). (Maclean Lake was also used as an external control for microbiological purposes in 1973.) All lakes were situated on the Precambrian Shield.

Harp was a softwater lake approximately 10 km northeast of Huntsville with an area of 0.6 km^2 and a maximum depth of 37 m. The underlying geology of Harp Lake was banded biotite-migmatite which has been moderately (30 - 50%) granitized.

Maclean Lake, 9 km northeast of Coldwater, has an area of 1.0 km^2 and a maximum depth of 10.8 m. Situated close to the edge of the shield (within 2 km), Maclean Lake lay over sedimentary rock which was predominantly metamorphosed and some Black River limestone complex. Neither Maclean nor Harp Lake were affected by heavy metals or acid mine wastes.

Minnow Lake, which was within the city limits of Sudbury, lay less than 0.8 km north of Ramsey Lake over three distinct

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geological features: Ramsey Lake conglomerate, gabbro and metagabbro (igneous), and McKim pelites. The latter was a very fine grain sedimentary rock containing mostly clay, minute quartzite and may be found with or without carbonates. This allowed for an inherent buffering capacity. Minnow Lake was small and relatively shallow (area 0.3 km^2, 3.5 m maximum depth) and at one time received sewage effluents, sawmill and domestic wastes. Consequently, the nutrient content was quite high allowing for a high population of algae throughout the sampling season (June to October).

The geological characteristics of the Reclamation Lakes were quite different from the external controls. Hannah Lake was completely bounded by metasediments of the Wanapitai quartzite formation. The geology of Middle Lake was similar but with gabbro and metagabbro (mafic intrusions) on the west shoreline. The northwest shore of Lohi was also gabbro/metagabbro with Wanapitai quartzite south of the lake. Clearwater, which straddles the Grenville Front, was bounded by gabbro/metagabbro to the northwest, Wanapitai quartzite to the north, and Grenville gneiss, migmatite and granite intrusions to the southeast. Morphometric descriptions of the Reclamation Lakes may be found in J. Adamski, M. Paylor, & W. Scheider; Reclamation of Acidified Lakes near Sudbury, Ontario; (in press).

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I.2 Methods:

Microbiological samples on the Reclamation Lakes were taken at approximately three week intervals from early June until mid-October (1974) by M.O.E. Water Resources Branch personnel from Limnology and Toxicity. Two stations per lake were monitored at surface (1 m below surface), depth (1 m above bottom) and sediment levels. All samples taken on Harp, Maclean and Minnow Lakes were done by Microbiology staff. Usually two stations per lake were monitored at the surface, depth (where possible), and sediment levels.

Surface water samples were taken by attaching a sterile 250 ml polycarbonate bottle to a 3.0 m aluminum pole. Depth samples were taken with a modified piggy-back sampler which filled a sterile, evacuated 250 ml rubber bulb. All sediment samples were taken with a sediment corer, and the top 4-5 cm of the core were decanted into a sterile 125 ml polycarbonate jar. Samples were transported to the Toronto laboratory on ice and analysed within 24 hours of sampling.

Water samples were routinely analysed for heterotrophic organisms (heterotrophic plate count - HPC), acid-tolerant (aciduric plate count - APC), and yeasts. Levels of blue-green algae, sulphate reducers, sulphur oxidizers and ammonia oxidizers were also periodically examined. Sediments were tested for aerobic and anaerobic heterotrophs, and occasionally for sulphate (SO_4) reducers, sulphur (S) oxidizers and ammonia (NH_4) oxidizing bacteria.

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Plate counts were obtained by using a spread plate technique in which 0.1 ml of the sample (or diluted sample) was plated onto duplicate predried plates (50 X 12 mm) and spread with a sterile glass rod. The HPC medium was a modified Foot and Taylor Agar (see Appendix I, Media Formulations), pH 7.2. To analyse for APC, the same medium acidified to pH 4.6 (0.1 N HCl) was used. Actidione (cycloheximide) was added to both media at a concentration of 150 ppm to reduce yeast and mold contaminations. Plates were incubated at 20°C for 7 (HPC) and 20 (APC) days.

Yeast and blue-green algae were determined by membrane filtration (MF) methods. The former was incubated on acidified (pH 4.0, 10% lactic acid) malt yeast agar for 3 days at 20°C. The method employed for blue-green algae isolation was that of McCurdy & Hodgson (1973), using BG-11 agar.

The sulphur and nitrifying bacteria were enumerated using the most probable number (MPN) method, with triplicate replication for each dilution. Sulphate reducers were analysed using API with tryptone incubated anaerobically at 20°C for 3-4 weeks. Sulphur oxidizers (acidophilic and non-acidophilic) were enumerated using Postgate's (1966) formulation, and incubated at 20°C for 3-4 weeks. Ammonia oxidizers were also incubated at 20°C for 4 weeks. Media descriptions for MPN methods can be found in Appendix I, Media formulations.

Sediment samples were added to sterile phosphate (PO_4) buffered dilution blanks by adding either 10 g in 90 ml or 1 g

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in 99 ml, depending on the dilution required. The diluted sample was homogenized for 15 seconds on a blender prior to analysis. All results were converted to a dry weight (count/g dry weight) basis.

In early October, certain lake samples were filtered and extracted for ATP following the method of Holm-Hansen and Booth (1966). After extraction in boiling TRIS (0.05 M, pH 7.5) buffer, all samples were frozen at -30°C until analysis was convenient. Aliquots (0.2 ml) were mixed with 0.4 ml luciferin/luciferase, and the counts per minute (CPM) were recorded on a Lab-line ATP photometer. The CPM values were then converted to ATP concentrations (ug/ml) by reference to a standard ATP curve.

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I.3 Results:

As sampling occurred at three (3) week intervals, accurately tracing population differences because of normal seasonal variations was difficult. Consequently, seasonal fluctuations were ignored and all data, for each parameter, was pooled for statistical purposes. On the basis of the Multiple "t" Test, checks were made for significant differences between stations at either the surface of depth levels. Some "between" level differences were observed. The data was then treated on a whole lake basis to obtain a geometric mean (GM) for each parameter at the different depths in the lake. This is summarized in Figures 1.1 and 1.2.

Lake to lake comparisons, which tested for significant differences, were then completed (see Tables 1.5-8 , Appendix II, Statistical Summaries). The treated lakes approximated the eutrophic controls and the untreated lakes were similar to the oligotrophic control. Using GM values, heterotrophic-aciduric (HPC:APC) bacterial ratios were calculated for the surface and depth waters for each lake (Table 1.1). Because of variations in sediment samples, each station per lake was treated as an autonomous unit for statistical analysis. See Figures 1.3 and 1.4.

Some data may not reflect expected trends because of high variability. This problem can be partially eliminated by increased sampling effort in 1975.

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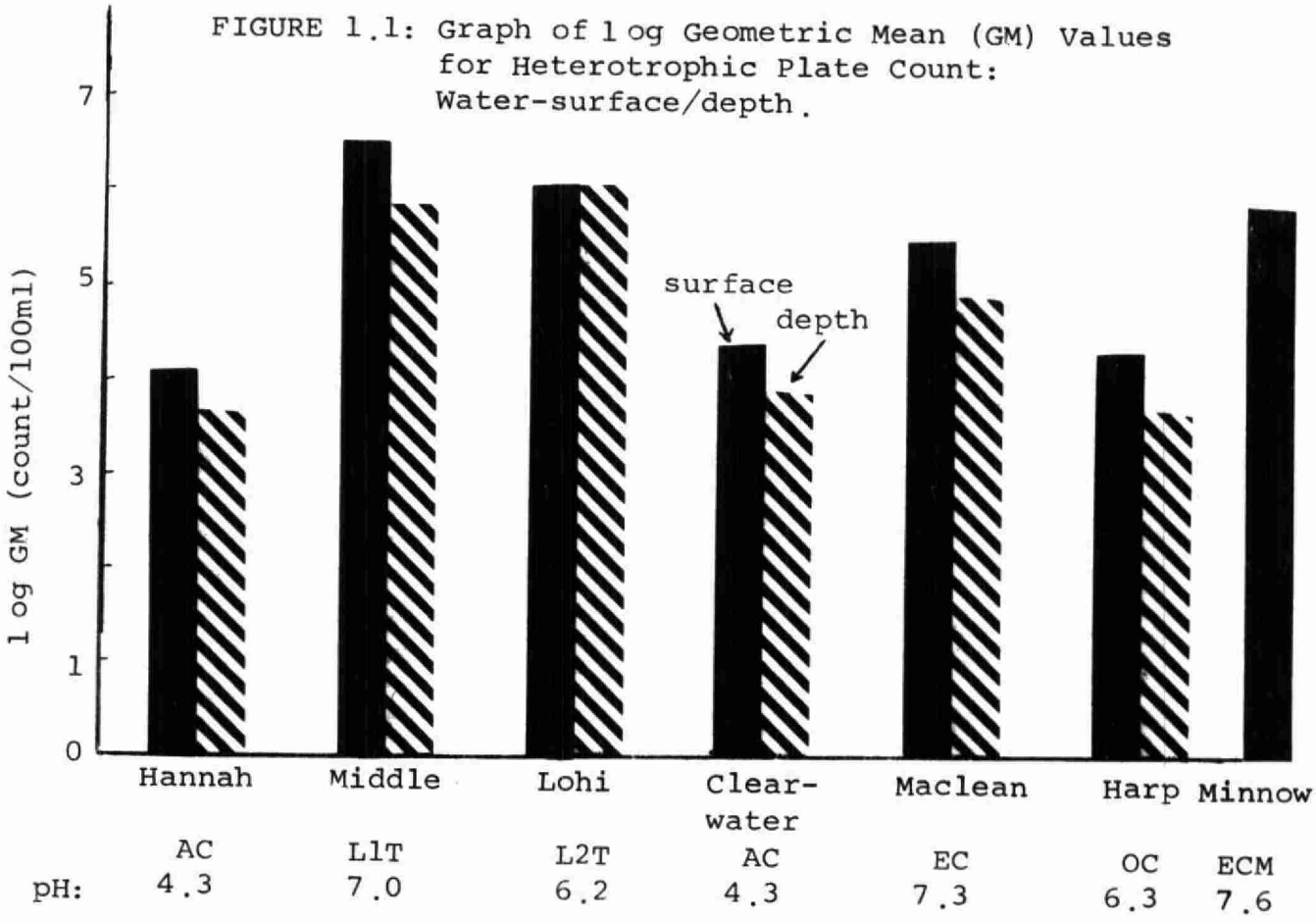
FIGURE 1.1: Graph of log Geometric Mean (GM) Values for Heterotrophic Plate Count: Water-surface/depth.



KEY:

Hannah Lake: acid control (AC) for Middle Lake.

Middle Lake: treated with $CaCO_3/Ca(OH)_2$ (L1T).

Lohi Lake: treated with $Ca(OH)_2$ only (L2T).

Clearwater Lake: acid control (AC) for Lohi Lake.

Maclean Lake: eutrophic control (EC), Precambrian Shield.

Harp Lake: oligotrophic control (OC), Precambrian Shield.

Minnow Lake: eutrophic control and heavy metal influence (ECM), Precambrian Shield.

NOTE: The above key applies to all subsequent graphs.

FIGURE 1.2: Graph of log Geometric Mean (GM) Values for Aciduric Heterotrophic Plate Count: Water-surface/depth.

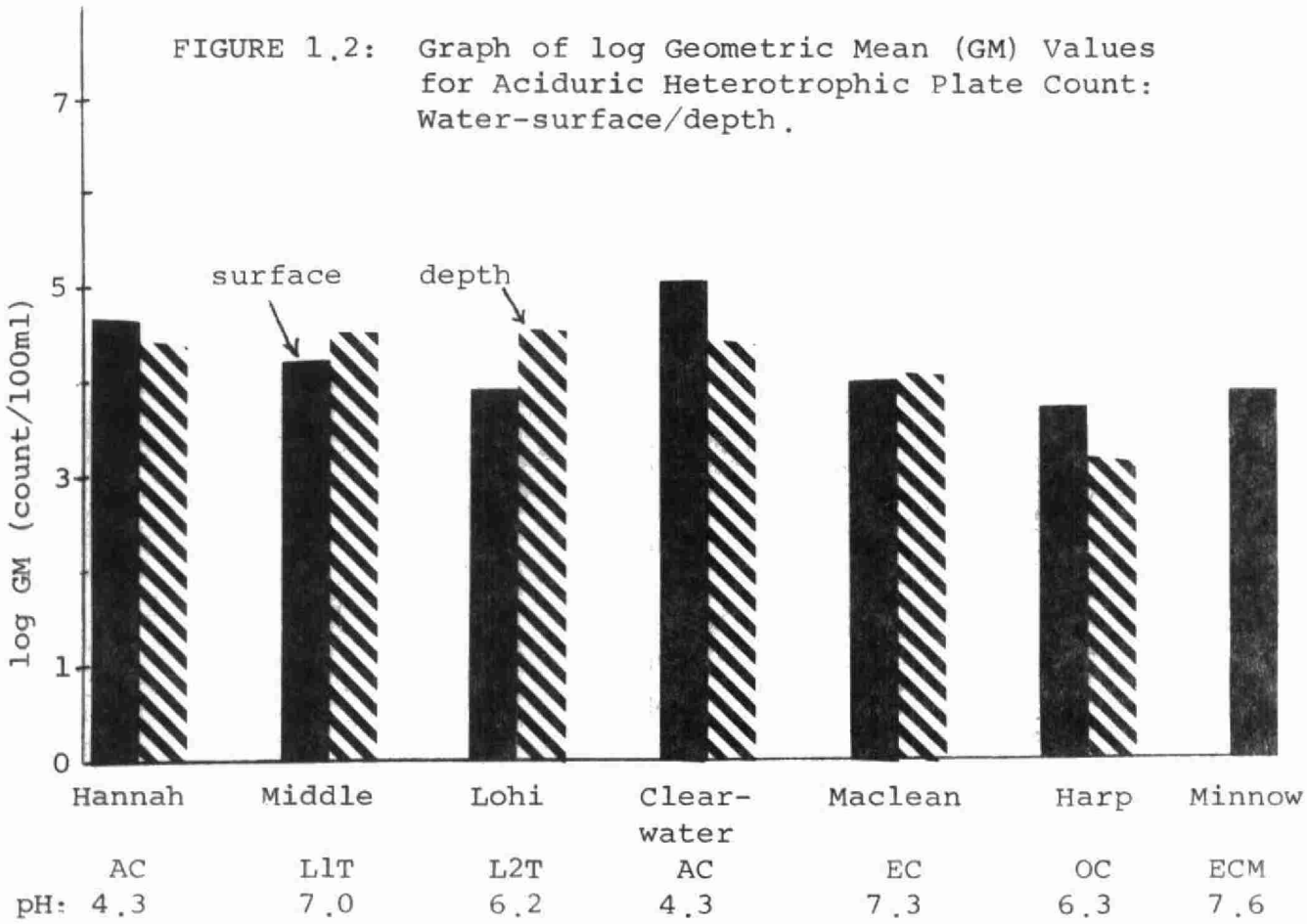


TABLE 1.1 Heterotrophic: Aciduric Plate Count (HPC: APC) Ratios Based on Geometric Mean Values for Water- surface and depth.

	HPC: APC Ratio	
LAKE	Surface	Depth
Hannah	0.27	0.18
Middle	184.0	20.27
Lohi	136.5	26.32
Clearwater	0.18	0.28
Maclean	28.75	5.91
Harp	3.13	2.75
Minnow	91.02	37.04

FIGURE 1.3: Graph of log Geometric Mean (GM) Values for Sediment Aerobes and Anaerobes.

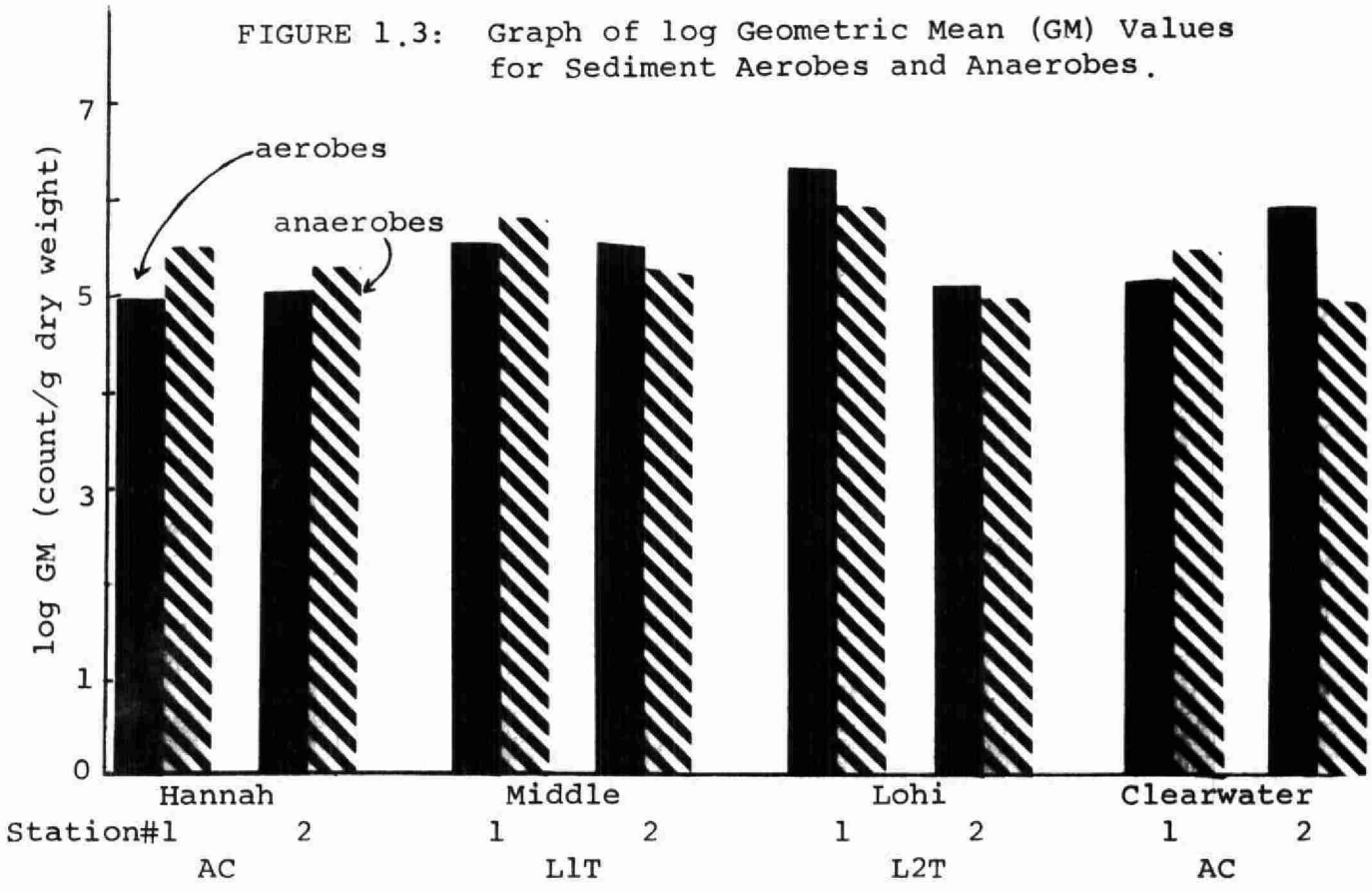
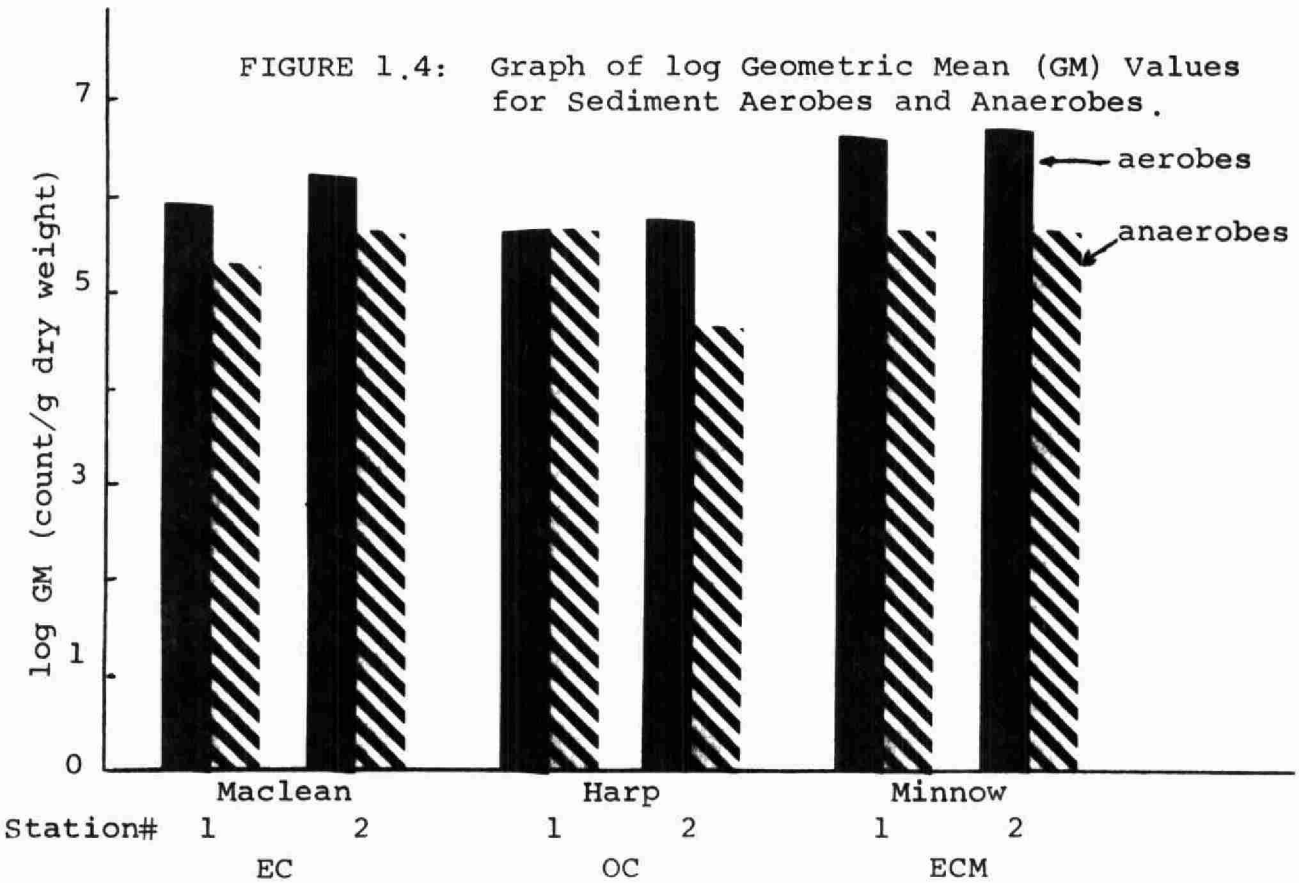


FIGURE 1.4: Graph of log Geometric Mean (GM) Values for Sediment Aerobes and Anaerobes.



Comparison of sediment data did not yield any significant trends in either aerobic or anaerobic (Appendix II, Table 1.9 and 1.10) bacterial populations. Sulphate reducers, infrequently analysed, did not yield a high population except in the eutrophic lakes Maclean and Minnow (Figure 1.5; Appendix II, Table 1.11).

Sulphur oxidizing (Thiobacillus sp.) and ammonia oxidizing (Nitrosomonas) bacteria were also tested, but the counts were quite low. Because of the problems with media stability for the sulphur oxidizers, the data was considered questionable and was not used for lake quality evaluation (Appendix II, Table 1.12).

Levels of ammonia oxidizers (Appendix II, Table 1.13) were less than 10/g wet weight, except in Maclean Lake and Station 2 of Hannah Lake where the counts were 7.8×10^3 and 290/g wet weight respectively. These results were based upon one sampling in early September.

Blue-green algae determinations were made but later taxonomic work showed that the method was not completely selective. Many of the isolated colonies were green algae (e.g. Chlamydomonas, Chlorella). Figure 1.6 represented the algal population counted, which was not directly indicative of blue-green levels.

Determination of ATP was done once from filtered water samples from each lake. The filtration method was non-differential, in that the ATP extracted was from all microbial sources: bacterial, algal, fungal and protozoan. Therefore the results (Table 1.2) were indicative of total active biomass from all sources.

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FIGURE 1.5: Graph of log Geometric Mean (GM) Values for Sulphate Reducing Bacteria in Sediment.

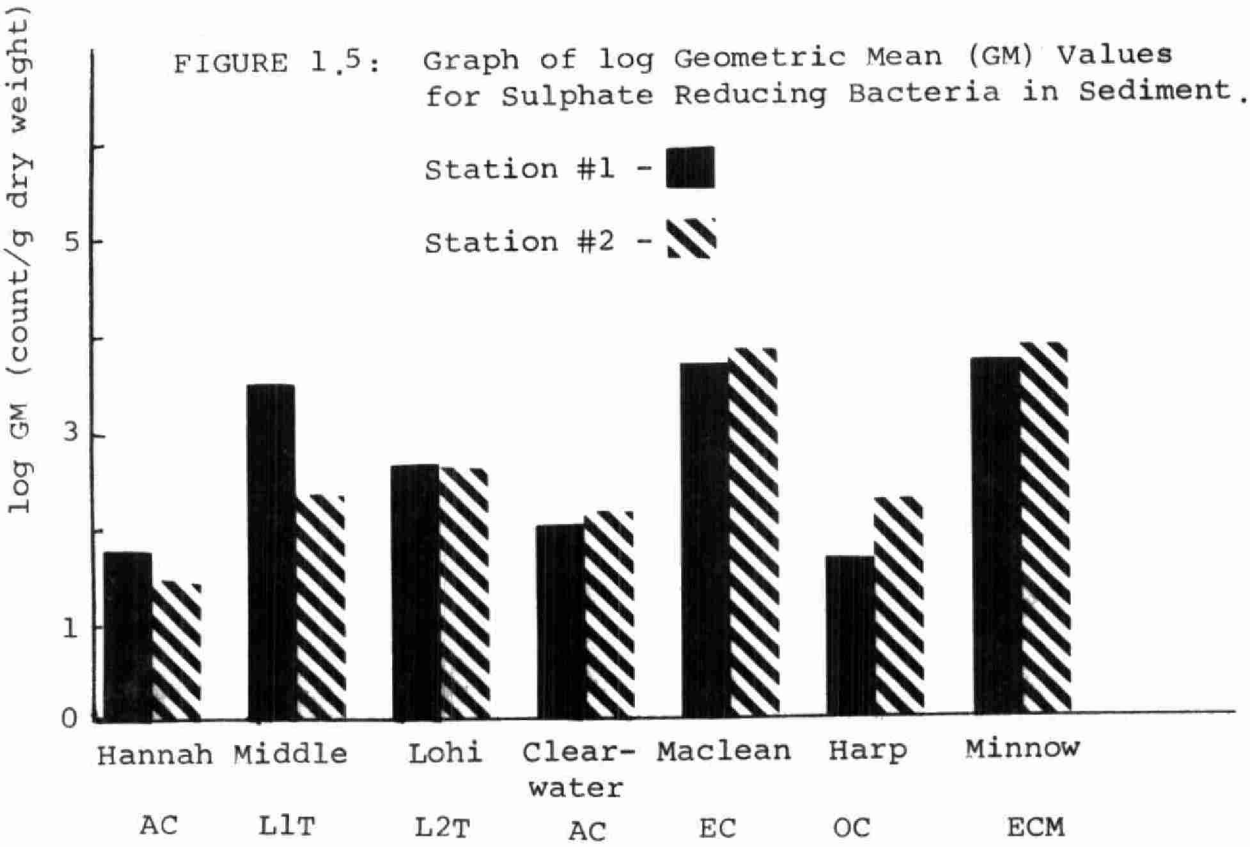

FIGURE 1.6: Graph of log Geometric Mean (GM) Values for Algal Levels in Surface Water.

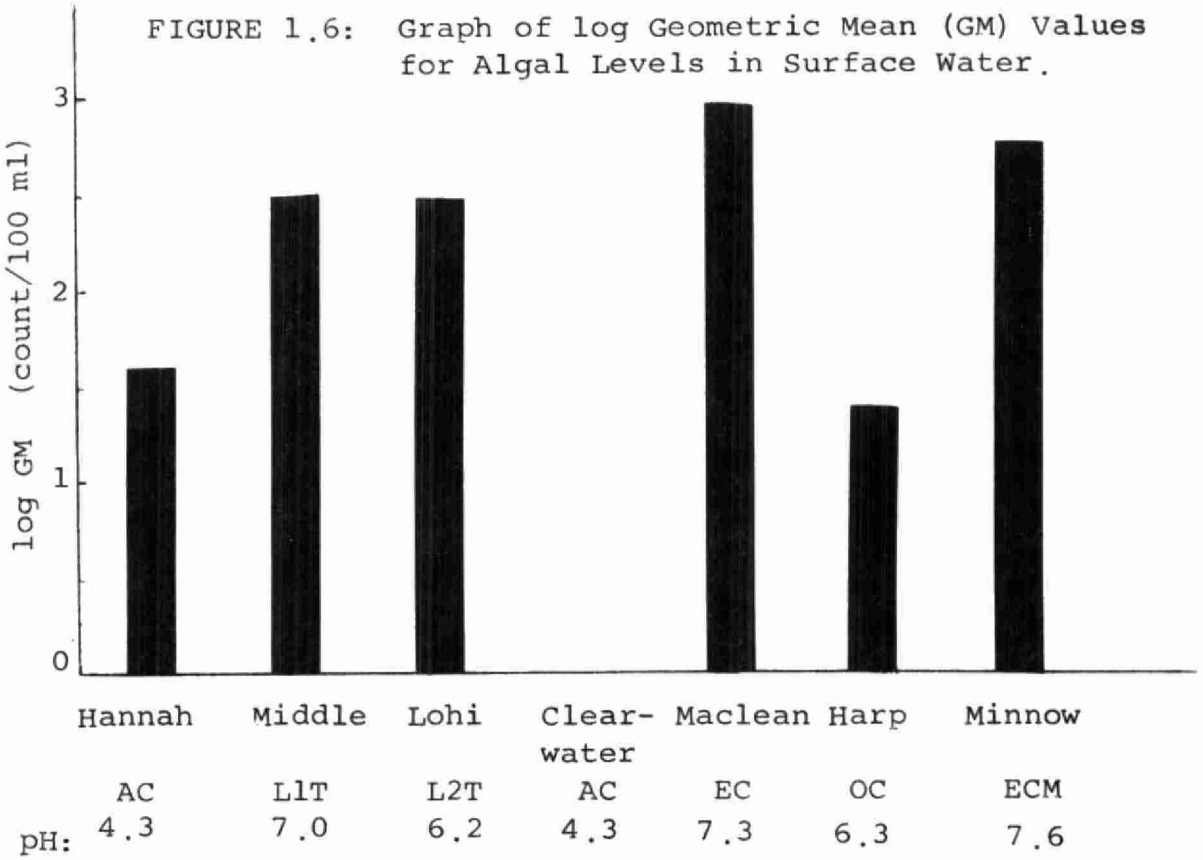

TABLE 1 ? : Summary of ATP Concentration (ug/ml), Calculated Bacterial Population (count/100 ml), and Heterotrophic Plate Count (HPC-count / 100 ml) in Lake Water.

LAKE	SURFACE			DEPTH		
		BACTERIAL POPULATION			BACTERIAL POPULATION	
	ATP conc.	Calculated*	HPC	ATP conc.	Calculated*	HPC
Hannah	4.80×10^{-5}	3.2×10^{6}	2.0×10^{4}	—	—	—
Middle	6.13×10^{-5}	4.1×10^{6}	7.9×10^{5}	—	—	—
Maclean	1.27×10^{-3}	8.4×10^{7}	6.0×10^{5}	5.17×10^{-4}	3.4×10^{7}	1.6×10^{5}
Harp	2.59×10^{-4}	1.7×10^{7}	3.6×10^{4}	8.45×10^{-5}	5.6×10^{6}	$<2.0 \times 10^{3}$
Minnow	3.13×10^{-3}	2.8×10^{8}	1.1×10^{6}	—	—	—

* Average ATP concentration / cell = 1.5×10^{-9} ug/ml (Holm-hansen & Booth, 1966)

Yeast levels on the average were low (<100/100 ml) and did not display noticeable seasonal variations. Counts apparently fluctuated at random, making statistical comparisons difficult (Table 1.3).

Micro-organisms are sensitive to the physical and chemical conditions of their habitat. Thus for proper interpretation of results, the physical-chemical data must also be considered (see Table 1.4). Chemical data for the Reclamation Lakes has been taken from summary sheets prepared by Water Resources Branch personnel, and averaged. Data for Harp, Maclean and Minnow Lakes is from sheets prepared by Microbiology staff. All samples were analysed in the Toronto laboratory.

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TABLE 1.3 : Summary of Geometric Mean (GM) Values for Yeast Populations in Water - surface & depth levels.

LAKE	SURFACE	DEPTH
Hannah	102 (10)*	17 (10)
Middle	53 (10)	24 (10)
Lohi	28 (8)	9 (8)
Clearwater	155 (6)	5 (6)
Maclean	18 (5)	21 (5)
Harp	16 (6)	3 (6)
Minnow	82 (5)	260 (1)

* GM (n)/100 ml

TABLE 1.4 : Physical- Chemical Data (Mean Values, 1974)- Lake Water.

LAKE	PARAMETER									
	Cu	Ni	Zn	Pb	Fe	Alkalinity (as $CaCO_3$)	pH	NH_4-N	org.-N	NO_3-N
Hannah	1.21	1.18	0.12	0.02	0.13	0.0	4.3	0.02	0.12	0.55
Middle	0.07	0.34	0.03	0.01	0.10	6.3	7.0	0.05	0.14	0.40
Lohi	0.04	0.19	0.03	0.01	0.05	2.2	6.2	0.02	0.10	0.08
Clearwater	0.20	0.29	0.05	0.01	0.10	0.0	4.3	0.02	0.07	0.09
Harp	0.04	<0.04	0.03	--	<0.03	8.9	6.3	0.09	0.14	0.01
Maclean	0.03	0.03	0.02	--	0.29	25.0	7.3	0.11	0.63	0.02
Minnow	0.09	0.13	0.02	0.01*	0.32	35.3	7.6	0.03	0.58	0.02

N.B.- Units (unless otherwise noted are ppm (mg l^{-1})

* - single piece data

TABLE 1.4 (cont'd) : Physical - Chemical Data for Lake Water.

LAKE	PARAMETER								
	Tot. P	Sol. P	Mg	Ca	K	SO_4^{2-}	Cl^-	Conductivity (umhos/cm)	Chlorophyll a ($\mu g\ l^{-1}$)
Hannah	0.005	0.002	3.6	11.0	2.1	56.8	2.6	166	0.4
Middle	0.007	0.002	2.5	14.2	1.7	41.7	1.5	217	0.9
Lohi	0.006	0.003	1.3	7.8	1.0	25.6	1.6	72	0.8
Clearwater	0.004	0.002	1.3	5.5	1.0	25.5	1.2	86	0.5
Harp	0.009	0.002	<1.0*	3.0*	0.9*	9.0*	0.9	36	2.0
Maclean	0.031	0.005	1.0	8.0	1.1	6.0	1.5	96	7.8
Minnow	0.041	0.003	5.8	27.8	3.1	40.0	136.0	611	9.0

N.B. - Units (unless otherwise noted) are ppm ($mg\ l^{-1}$)

* - single piece data

1.4 Discussion:

In spite of some high variance values used in the Multiple "t" Test, a definite pattern emerged for the heterotrophic (HPC) data in the water column (Table 1.5 & 1.6, Appendix II). The untreated acid lakes (Clearwater, Hannah) for this parameter, were significantly different (at the 99% confidence limit) from the treated lakes and the eutrophic controls, Maclean and Minnow. However, no differences in the counts of the untreated lakes and the oligotrophic control (Harp) at either surface or depth levels were observed.

The aciduric data (AppendixII, Table1.7,8) was much more variable and did not produce the consistent pattern that was shown for the HPC results for the surface and depth levels. Differences among the lakes studied was better demonstrated by the HPC : APC ratios (Table 1.1). The acid lakes had values less than 1.0 at both surface and depth, while all other lakes had values much greater than 1.0. The HPC : APC ratios for the limed lakes (Lohi, Middle) usually exceeded those of the oligotrophic and eutrophic controls.

Data collected in 1973 demonstrated that heterotrophic populations in each pair of lakes (Hannah-Middle, Clearwater-Lohi) were equivalent prior to lime treatment. Post-treatment evaluation of the water quality revealed sharp increases in the heterotrophic populations of the limed lakes (Middle - calcium carbonate/calcium hydroxide; Lohi - calcium hydroxide only) as well as pH neutralization (Refer to Microbiology Report on SES, 1973).

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These effects of lime treatment were maintained in 1974. (Because of methodology changes, a direct comparison of 1973 and 1974 microbiological results could not be made.) Further increases in bacterial levels may still occur. Increased frequency of sampling in 1975 may establish this possibility.

Lime treatment effects were not apparent in the sediment data because of natural lake variability (ref. Adamski et al.). Results indicate that the sediments have not yet responded, in a distinct manner, to treatment. The observed effects in the water column were probably because of improved pH conditions and not necessarily because of nutrient release from the sediments. This was supported by physical-chemical data (Table 1.4).

Because of geochemical and physical-chemical characteristics, the Reclamation Lakes had inherently low nutrient levels and naturally poor buffering capacities. The bedrock underlying these lakes was predominantly quartzite. The major chemical constituent of quartzite is silicon dioxide (SiO_2) which does not have the buffering capability of a carbonate system. The probability of natural eutrophication of these lakes was quite low.

Water column heterotrophic population increases may have been the result of aggregation of dissolved organic carbon (D ') on small particles which were affected by pH and probably calcium ion concentrations (Lush and Hynes, 1973). Several literature reports were made emphasizing the importance of

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organic matter-microbial complexes in water ecosystems (Cummins et al, 1972; Paerl, 1974; Pomeroy, 1974). Pomeroy (1974) gave evidence that the primary consumers of DOC were bacteria and the phytoplankton were the metabolically dominant system in water. The re-establishment of the bacteria in the Reclamation Lakes was an initial requirement before increases of other organisms in the food chain could be observed.

Sulphate reducer populations were only found at significant levels in Maclean and Minnow Lakes and at Station #1 of Middle Lake. Data variability was high in most cases (Appendix II, Table 1.11). The SO_4 reducer population was only a minor contributor to bacterial biomass in the sediments. Conditions may not have been suitable for optimal growth of these obligate anaerobes. Sulphur oxidizer levels were, on the average, higher. Because of media instability (previously noted) the results were questionable and not used in lake quality evaluation.

Analysis for ammonia oxidizing bacteria, conducted once during the season, was eliminated because of low levels. When a "liming effect" is observed in the heterotrophic sediment bacterial populations, re-introduction of tests for these organisms would be advisable.

Differences in algal populations between the treated and and untreated lakes were observed. One of the untreated lakes, Hannah, had GM values greater than the oligotrophic control, Harp Lake. The eutrophic control lakes, Maclean and Minnow, had higher populations than the treated lakes. Exact values

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for Minnow Lake were not obtained because all plates were too numerous to count (TNTC). A number of cultures were isolated and identified as blue-greens, predominantly various species of Oscillatoria.

Holm-Hansen & Booth (1966) reported the average ATP content of a bacterial cell as 1.5×10^{-9} ug ATP. On this basis, a theoretical number of bacteria/100 ml was calculated (Table 1.2). In all cases, this number was higher than the level recorded by the plating method on the same sample. Because only the cells that can grow under the conditions provided will be recovered by a plating technique, the viable count should be a low estimate of total microbial biomass (Holm-Hansen & Booth, (19 66).

Low populations of yeasts were found in all lakes (Table 1.3), but GM levels in surface water were greater than in depth samples. Yeasts were unsatisfactory as indicator organisms, and no analysis for them will be made in 1975.

1.5 Conclusions:

1) The "liming effect" was maintained in the water column in 1974.

2) Sampling effort should be increased in 1975 so that seasonal variations may be traced and data variability eliminated.

3) Effort should be concentrated on sediment population dynamics and how this affects water column microbial levels.

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4) Continued study of blue-green algae, with improved isolation techniques, would be beneficial in order to measure N_2-fixation potential of the treated lakes.

5) Use of the ATP method for total biomass determinations should be continued because of its high sensitivity.

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APPENDIX I - MEDIA FORMULATIONS

(a) Foot and Taylor (Modified) Agar

peptone	3.0 g
K_2HPO_4	0.2 g
$MgSO_4$	0.05 g
$FeCl_3$	trace
casein	0.05 g
agar	20.0 g

1000 ml distilled H_2O
pH - 7.2

(b) Sulphate Reducing Bacteria - API with tryptone Broth

yeast extract	1.0 g
tryptone	3.0 g
sodium lactate	5.2 g
$MgSO_4$	0.2 g
K_2HPO_4	0.01 g
Ascorbic acid	0.1 g *
$FeNH_4(SO_4)_2$	0.1 g *

1000 ml distilled H_2O
pH - 7.5

* add prior to inoculation along with sterilized iron nail.

(c) Ammonia Oxidizing Bacteria - Nitrosomonas Broth

$(NH_4)_2SO_4$	2.0 g
K_2HPO_4	2.0 g
NaCl	1.0 g
$MgSO_4$	1.0 g
$CaCl_2$	0.2 g
Trace element solution	1.0 ml/l
$CaCO_3$	1.0 g

1000 ml distilled H_2O
pH - 7.4

(d) Sulphur Oxidizing Bacteria

i) Acidophilic

Thiooxidan Broth

$Na_2S_2O_3 \cdot 5H_2O$	5.0 g
NH_4Cl	1.0 g
KH_2PO_4	3.0 g
$CaCl_2$	0.1 g
$MgSO_4$	0.5 g
trace element solution	1.0 ml/l.

1000 ml distilled H_2O
pH - 4.5

ii) Non-Acidophilic

Thioparus Broth

$Na_2S_2O_3 \cdot 5H_2O$	5.0 g
NH_4Cl	1.0 g
K_2HPO_4	3.0 g
$CaCl_2$	0.1 g
$MgSO_4$	0.5 g
trace element solution	1.0 ml/l.

1000 distilled H_2O
pH - 7.2

Table 1.5 Statistical Results of Lake to Lake Comparisons for surface Water HPC Geometric Mean Values.

	Clearwater	Lohi	Hannah	Middle	Maclean	Harp	Minnow
Clear-water		SD*	-	SD*	SD*	-	SD*
Lohi			SD*	-	-	SD*	-
Hannah				SD*	SD*	-	SD*
Middle					SD*	SD*	SD
Maclean						SD*	-
Harp							SD*
Minnow							

Table 1.6 Statistical Results of Lake to Lake Comparisons for depth Water HPC Geometric Mean Values.

	Clearwater	Lohi	Hannah	Middle	Maclean	Harp	Minnow
Clear-water		SD*	-	SD*	SD*	-	SD*
Lohi			SD*	-	SD*	SD*	-
Hannah				SD*	SD*	-	SD*
Middle					SD	SD*	-
Maclean						SD*	SD
Harp							SD*
Minnow							

Table 1.7 Statistical results of Lake to Lake Comparisons for surface Water APC Geometric Mean Values.

	Clearwater	Lohi	Hannah	Middle	Maclean	Harp	Minnow
Clear-water		SD*	-	SD	SD*	SD*	SD
Lohi			-	-	-	-	-
Hannah				-	-	SD	-
Middle					-	-	-
Maclean						-	-
Harp							-
Minnow							

Table 1.8 Statistical Results of Lake to Lake Comparisons for depth Water ACP Geometric Mean Values.

	Clearwater	Lohi	Hannah	Middle	Maclean	Harp	Minnow
Clear-water		-	-	-	-	SD*	-
Lohi			-	-	-	SD*	-
Hannah				-	-	-	-
Middle					SD	SD*	-
Maclean						SD*	-
Harp							SD
Minnow							

Table 1.9 Statistical Results of Lake to Lake Comparisons for Sediment Aerobe Geometric Mean Values.

	Clearwater 1	Clearwater 2	Lohi 1	Lohi 2	Hannah 1	Hannah 2	Middle 1	Middle 2	Maclean 1	Maclean 2	Harp 1	Harp 2	Minnow 1	Minnow 2
Clear-water 1		SD	-	-	-	-	-	-	-	-	-	-	-	-
2			SD*	-	-	-	-	-	-	SD	-	-	SD	SD
Lohi 1				SD	SD	SD	SD	-	-	-	SD	SD	-	-
2					-	-	-	-	-	-	-	-	SD*	SD
Hannah 1						-	-	-	-	-	-	-	-	-
2							-	-	-	-	-	-	SD	-
Middle 1								-	-	-	-	-	SD	SD
2									-	-	-	-	-	-
Maclean 1										-	-	-	-	-
2											SD	SD	-	SD*
Harp 1												-	SD	SD
2													SD	SD*
Minnow 1														-
2														

Table 1.0 Statistical Results of Lake to Lake Comparisons for Sediment Anaerobe Geometric Mean Values.

	Clearwater		Lohi		Hannah		Middle		Maclean		Harp		Minnow	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Clear-water 1		-	-	-	-	-	-	-	-	-	-	-	-	-
2			SD	-	SD*	-	SD*	-	-	SD	SD	-	SD*	SD
Lohi 1				-	-	-	-	-	-	-	-	SD	-	-
2					-	-	SD*	-	-	-	-	-	-	-
Hannah 1						-	-	-	-	-	-	SD*	-	-
2							-	-	-	-	-	-	-	-
Middle 1								-	-	-	-	SD	-	-
2									-	-	-	-	-	-
Maclean 1										-	-	-	-	-
2											-	SD	-	-
Harp 1												-	-	-
2													-	SD
Minnow 1														-
2														

Table 1.11 Summary of log Geometric Mean (GM) Variance (S^2), n and GM values for Sulphate Reducers.

Lake (Stn)	log GM	S^2	n	GM (c/g dry wt)
Hannah 1	1.7706	0.2958	3	59
2	1.4683	0.0907	2	29
Middle 1	3.4974	0.2244	3	3143
2	2.3817	1.9356	2	241
Clearwater 1	2.0500	0.2861	3	112
2	2.1695	0.8826	2	148
Lohi 1	2.6853	1.0389	3	484
2	2.6198	4.7469	2	417
Maclean 1			1	6111
2			1	7333
Harp 1			1	48
2			1	288
Minnow 1	3.8139	0.0053	2	6515
2			1	7586

Table 1.12 Summary of log Geometric Mean (GM), Variance (S^2), n and GM values for Sulphur Oxidizers.

Lake (Stn)	log GM	S^2	n	GM (c/g dry wt)
Hannah 1	2.5339	3.3978	2	340
2	2.5603	3.4061	2	360
Middle 1	3.4181	0.9628	2	2620
2	2.2828	0.0204	2	190
Clearwater 1			1	188
2			1	682
Lohi 1	4.0754	0.0003	2	11900
2	3.0787	0.6579	2	1200
Maclean 1			1	517
2			1	1600
Harp 1			1	307
2			1	38
Minnow 1			1	147
2			1	186

Table 1.13 Summary of Ammonia Oxidizer Populations in Lake Sediments (count/g wet weight)

Lake (Stn)	Count/g wet weight
Hannah 1	3
2	290
Middle 1	<3
2	9
Lohi 1	<3
2	4
Clearwater 1	<3
2	<3
Maclean 1	7.8×10^3
Harp 1	4

- SUMMARY -

Section 2.

An investigation of the persistence of certain natural organic materials and the capacity for heterotrophic productivity using some naturally-occurring polysaccharides as carbon sources, formed part of a study involving reclamation experiments on acid lakes in the Sudbury district.

A pair of oligotrophic lakes, Hannah and Middle located a few kilometres south of the Copper Cliff smelting complex were compared with nearby Minnow Lake, chosen as an internal eutrophic control.

Two lakes not influenced by local industrial operations were chosen as external controls. Both lakes, in the Muskoka district of Ontario were on the Precambrian shield, as are the Sudbury lakes, but Harp L. was oligotrophic and Maclean L. was eutrophic.

An attempt was made to determine the degradation rates of cellulose, chitin and birch leaves, contained in nylon fabric bags and placed in the lake epilimnion in early August. Substrate bags were collected and taken to the laboratory for analysis at various intervals over a three month period.

Examination of the nylon bags and organic substrates in the eutrophic Minnow and Maclean Lakes, indicated that the surface of these materials were coated with greenish-brown microbial slime (periphyton). Less surface-attached growths were observed in the oligotrophic Middle and Harp Lakes. In the acidic

oligotrophic Hannah Lake, no microbial slime accumulation was observed on the substrates. Because of differences in microbial slime attachments, precise determination of substrate weights was impossible.

To determine the potential for heterotrophic productivity, dialysis sacs containing pre-concentrated lake water alone, and treated with the three carbon sources, chitin, xylan and starch were placed in the epilimnion of each lake. A set of dialysis sacs from each treatment was collected at bi-weekly periods and taken to the laboratory for analysis of: heterotrophic bacteria, aciduric bacteria, yeast count and ATP determination.

The population of aerobic heterotrophic bacteria responded positively to chitin, hemicellulose and starch added to dialysis sacs. The increased bacterial growth response to chitin and xylan as compared to starch may be attributed to nitrogen and phosphate impurities associated with the former two substrates.

The acid-tolerant heterotrophs showed a marked response to all three carbon sources. These substrates caused greatest stimulation of aciduric bacterial growth in Hannah Lake compared to the other lakes.

Polysaccharide additions caused a greater stimulation of bacterial populations in the oligotrophic lakes than in the eutrophic lakes. This would indicate that organic carbon is a

limiting factor to the heterotrophic microbial population of the oligotrophic lakes.

Yeast populations in all lakes were low and remained unaffected by addition of carbon sources.

The ATP values reflected the stimulated increase in biomass because of the presence of the carbon substrates. As observed with bacterial counts, a significantly greater percentage increase in ATP concentration because of added carbon source was observed in dialysis sacs from Hannah Lake than in the sacs for controls or other lakes.

EXPERIMENTS ON THE MICROBIAL DEGRADATION OF NATURAL ORGANIC SUBSTRATES AND HETEROTROPHIC PRODUCTIVITY IN LAKES.

2.1 Introduction:

As part of a study involving lake reclamation experiments in the Sudbury area, investigations were undertaken to determine differences in decomposition rates of certain organic substances. The lakes selected were: Hannah, Middle, Minnow, Harp and Maclean.

In the first part of this study, the rates of degradation of cellulose, chitin particles and leaf litter were examined in the various lakes. The natural degradation of cellulose and dead leaves in aquatic environments has been studied by other workers using nylon fabric bags (Hofsten & Edberg, 1972; Kaushik & Hynes, 1971).

Cellulose is the most abundant organic compound distributed in the plant kingdom. Next to cellulose, starch and hemicellulose are the most common polysaccharides found in plants. Xylan, a common hemicellulose, and starch are more readily degraded than cellulose (Alexander, 1961).

Chitin, another water-insoluble polysaccharide abundant in nature, is found in the exoskeletons of arthropods and in the cell walls of some fungi. Unlike the other polysaccharides which serve as carbon sources only to microbial decomposers, chitin can be utilized as a nitrogen source.

Many species of bacteria, actinomycetes and fungi in soil and water are capable of breaking down these natural polysaccharides (Alexander, 1961).

The second part of the study was concerned with the determination of the relative potential of each lake to support heterotrophic microbial populations, when an organic carbon source was non-limiting. For these experiments, a specialized dialysis sac culture technique was employed. This method, based on the theory of Schultz & Gerhardt (1969), has been employed by other workers for studying the growth and survival of enteric bacteria in water (Hendricks et al, 1967, 1971; McFeeters & Stuart, 1972), and to measure heterotrophic activity in pond water (Hussenot & Laurent, 1973).

The dialysis sac freely permits the passage of gases, ions and soluble, low molecular weight organic compounds, but presents a barrier to high molecular weight polymers, microbial cells and particulate matter.

2.2 Materials and Methods:

Substrate degradation

A series of fine mesh, nylon fabric bags (5 X 10 cm) were filled with 0.5 grams of each of the following substrates:

(a) cellulose - Whatman No. 6 filter paper strips (1 X 3 cm)

(b) chitin - unbleached, Eastman practical grade, 10 mesh size

(c) birch leaves * - air-dried at 45 °C for 3 weeks.

* live, healthy, green leaves of native Canadian white birch (Betula papyrifera) were collected from a tree near Maclean Lake, Simcoe Co., Ontario in early July, 1974.

Duplicate nylon bags containing each substrate were placed in cages made of fiberglass window screening. Sufficient cages were made to permit retrieval from each lake at intervals of 2, 4, 8, 10 and 12 weeks.

These cages were positioned in the water as illustrated in Figure 2.1.

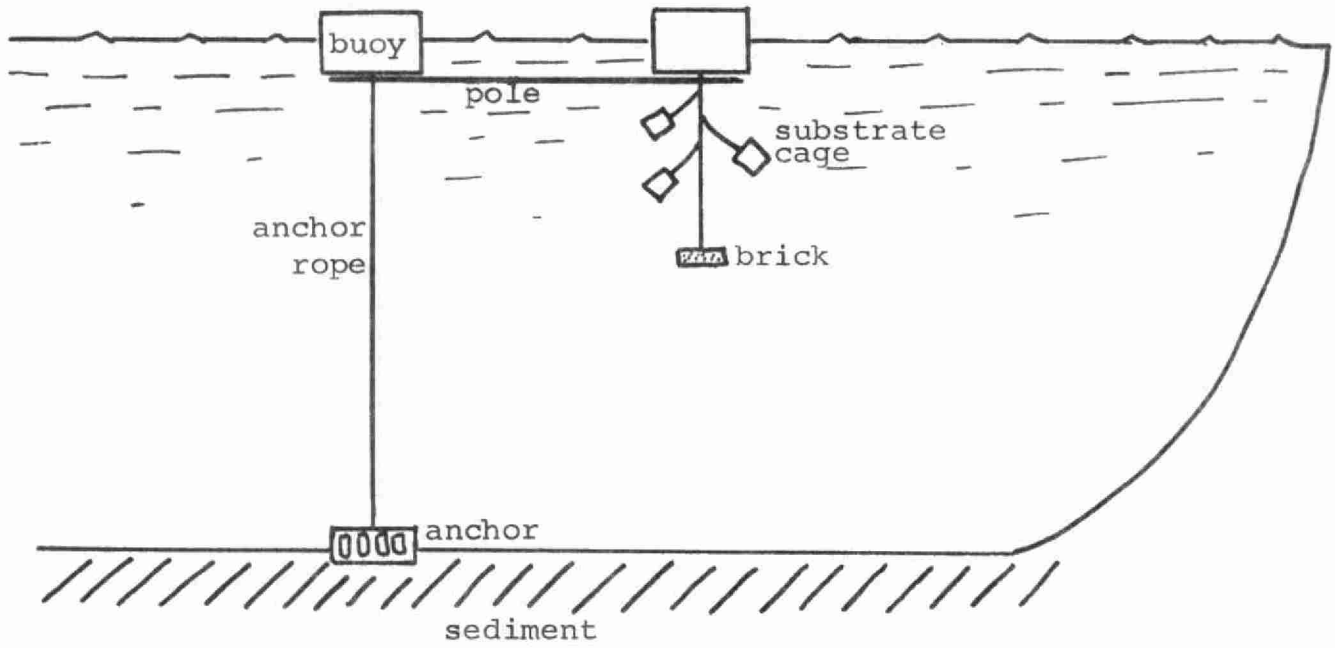

Figure 2.1 Diagram of experimental set-up in lakes.

Cages were placed in all lakes between August 7 and 14, 1974, when the surface water temperature was above 20 °C. Substrate bags were retrieved and taken to the laboratory at appropriate time intervals. Their contents were placed in glass petri dishes, dried to constant weight at 100 °C, and weighed to the nearest 0.01 g.

Heterotrophic production :

Surface water samples were collected from each of the lakes in early August. Microbial cells were concentrated on membranes (0.45 μ Millipore, plain), by filtering 200 ml of lake water where possible, through membranes, and aseptically cutting each membrane in half. Dialysis sacs were made from a roll of Fisher brand cellulose nitrate dialysis tubing (4.6 cm wide), by cutting 30 cm long sections which were soaked and washed in sterile water. One end of each tube was knotted and tied with nylon string, and 5.0 ml of a sterile aqueous suspension (1.5%) of each of the following substrates was placed into separate dialysis sacs: (a) chitin, unbleached, ball-milled, chemical reagent, Nutritional Biochemicals Inc., Cleveland, Ohio. (b) xylan, ball-milled, Nutr. Biochem., Cleveland, Ohio, biochemical reagent. (c) starch, colloidal, Analar reagent grade, (BDH).

A half section of a membrane filter was added to each dialysis sac and 25 ml water from the respective lake was also added which gave a final substrate concentration 2500 ppm. This procedure was carried out for each lake. Control sacs for each lake contained lake water and cells concentrated on filters without added substrate. The open end of each dialysis sac was knotted and tied with fine nylon string. Each sac was then suspended by the string inside a hollow 17.5 cm long cylinder of polyvinylchloride (PVC), having an inside diameter of 6 cm and a wall thickness of 0.5 cm.

As illustrated in Figure 2.1, cylinders containing dialysis sacs were tied to the rope at a depth of 1 m below the surface and incubated *in situ* from August to October in all lakes.

Dialysis bags for each of the treatments and controls were collected from the study lakes at 2, 4, 6, 8 and 10 weeks where possible. The PVC cylinders with the dialysis sacs still attached were placed in a plastic bag and bathed in lake water for transport to the Toronto laboratory in iced coolers. Analyses were performed on the sac contents within 24 hours of collection from the lake. The strings attaching the sac to the protective cylinder were cut; each dialysis sac was dipped in 70% ethanol and immediately washed under tap water. The sac was shaken well to mix the contents. One end of the sac was cut with sterile scissors and the contents transferred carefully into sterile wide mouth polycarbonate bottles.

The following analyses were performed to determine heterotrophic productivity:

(1) Heterotrophic Plate Count (HPC)

(2) Aciduric Plate Count (APC)

(3) Yeast count - membrane filter method

(4) Adenosine triphosphate (ATP).

The above methods were carried out according to the procedures described in Section 1 of this report.

(5) Nutrient analyses of substrates:

A check on possible nutrient impurities associated with

the starch, chitin and xylan employed as carbon sources in the dialysis experiments, was conducted. Protein (Lowry et al, 1951) and soluble carbohydrates (Morris, 1948) were analysed in acid and aqueous extracts (leachates) from each of the polysaccharide preparations. Total inorganic nitrogen and phosphorus were also determined on the leachates by the Water Quality Laboratory (M.O.E.).

2.3 Results and Discussion:

Substrate Degradation

Examination of the nylon bags and organic substrates in the eutrophic lakes, Minnow and Maclean, indicated that an appreciable accumulation of microbial slime (periphyton) had occurred on the solid surfaces. Significantly less periphyton was visible on substrate surfaces from the oligotrophic lakes, and likely accounted for some anomalies in residual dry weights of the materials recovered. Visibly, the three substrates, chitin, cellulose and birch leaves appeared to be in a more advanced state of decomposition in the eutrophic than in the oligotrophic lakes. Degradation of birch leaf material was more retarded in the acidic Hannah Lake than in the eutrophic lakes. Acid pH, high concentrations of copper and nickel and low nutrient levels in the water appeared to have a negative influence on decomposition of natural organic matter.

In this study, no attempt was made to enumerate or identify the various microorganisms which constituted the complex

community colonizing the substrates under investigation. The epilithic microbial communities attached to solid surfaces, referred to as periphyton or "aufwuchs" are made up of bacteria, fungi, actinomycetes and algae, and have been the subject of numerous investigations (Characklis, 1973; Collins, 1963; Cooke, 1956; Hendricks, 1974; Paerl, 1973).

The inhibition of periphyton accumulation in acid strip mine lakes has been reported by Stickney & Campbell (1972) who concluded that aufwuchs accrual on solid surfaces was indicative of biological production. Significantly less periphyton accumulation was observed on substrate surfaces in Hannah Lake.

With respect to cellulose decomposition in natural water, Hofsten & Edberg (1972) found that both bleached and unbleached cellulose was degraded more rapidly in polluted water where nutrient concentrations were high than in unpolluted open water. These authors concluded that the main growth limiting factor for cellulose decomposers was available nitrogen. The presence of toxic compounds and extreme pH also inhibit cellulose degradation.

Studies have been carried out which indicate that leaves entering natural waters may contribute to eutrophication (Cowen & Lee, 1973, Hall & Lee, 1974; Cummins et al, 1972; Wetzel & Manny, 1972). Recent evidence suggests a complex interaction between bacteria and fungi as primary decomposers of leaf litter and invertebrate grazers feeding upon these microorganisms (Brasdate et al, 1974). Kaushik & Hynes (1969, 1971) have

stressed the importance of allochthonous organic matter as a food source for microbes and invertebrates in water.

In the Sudbury study, decomposition of birch leaves was evident in all lakes as estimated by weight loss. Greater loss of the leaf material occurred from the neutral pH lakes than from the acidic Hannah Lake both after 6 and 12 weeks of incubation *in situ*.

Degradation of natural organic substrates proceeded in all lakes studied. Acid-tolerant microorganisms resident in Hannah Lake were capable of decomposing organic materials when available, but turn-over of organic carbon occurred at a slower rate than in the neutral pH lakes.

Heterotrophic production :

All substrates within the dialysis sacs were not completely utilized after the 10 week incubation period *in situ* in the lakes. As observed for the substrate bags, the lakes showing maximum accumulation of periphyton on the dialysis sac walls were Minnow and Maclean Lakes. As a result of microbial activity in these eutrophic lakes, the sacs broke open before 2 months incubation *in situ*. Thus, data for these lakes were based on analyses at 2, 4 and 6 weeks only. Relatively little slime accumulation was observed on the walls of the dialysis sacs in the oligotrophic lakes.

The effects of chitin, xylan and starch on populations of aerobic heterotrophic bacteria (HPC), aciduric heterotrophs (APC) and yeasts developing within dialysis sacs incubated in the study lakes were shown in Table 2.1. Geometric means of each parameter were calculated for each treatment based on data obtained from analyses at 2, 4, 6, 8 and 10 weeks where possible. (Individual data, is given in Appendix III, Tables 2.1 and 2.2; graphs depicting the microbial responses to the added carbon sources are also given in Appendix Figures 2.1, 2.2, 2.3 and 2.4.)

Aerobic heterotrophic bacteria (HPC) increased in response to chitin, xylan and starch in all lakes. The HPC geometric mean was always greater in the presence of added polysaccharide than in the respective controls. The geometric means were lower in Hannah and Harp Lakes than in Middle, Minnow and Maclean Lakes.

Table 2.1 - Development of microbial populations in dialysis sacs containing substrates incubated in various lakes.

PARAMETER & LAKE	CHITIN			XYLAN			STARCH		
	GM per 1 ml		Percent Increase Above Control	GM per 1 ml		Percent Increase Above Control	GM per 1 ml		Percent Increase Above Control
	Control	Chitin		Control	Xylan		Control	Starch	
Heterotrophic Plate Count (HPC)									
Hannah (acid cont)	5.2×10^3	6.3×10^5	60*	5.2×10^3	1.5×10^6	74*	2.2×10^3	4.8×10^5	72*
Middle (limed)	5.7×10^5	8.7×10^6	23*	5.7×10^5	2.5×10^7	29*	6.2×10^4	1.8×10^6	32*
Harp (olig)	8.2×10^3	4.0×10^6	60*	8.2×10^3	6.3×10^5	40*	4.0×10^4	6.2×10^5	28*
Minnow (eutr)	5.7×10^5	1.8×10^7	29*	5.7×10^5	1.5×10^7	25*	7.2×10^5	8.7×10^6	14
Maclean (eutr)	7.3×10^4	1.2×10^7	43*	7.3×10^4	2.6×10^7	45*	-	-	-
Aciduric Plate Count (APC)									
Hannah (acid cont)	2.4×10^2	4.6×10^5	150*	2.4×10^2	7.0×10^6	205*	2.2×10^2	5.2×10^5	144*
Middle (limed)	4.8×10^2	2.5×10^4	75*	4.8×10^2	1.8×10^5	110*	8.8×10^2	2.0×10^4	37*
Harp (olig)	1.7×10^3	4.8×10^4	41*	1.7×10^3	5.3×10^4	44*	9.3×10^3	5.1×10^4	18*
Minnow (eutr)	4.9×10^4	5.0×10^4	2	4.9×10^4	1.3×10^5	11	5.0×10^3	3.6×10^4	25*
Yeast Count									
Hannah (acid cont)	4.7	150	64*	4.7	7.2	8	4.9	6.8	23
Middle (limed)	2.1	3.0	10	2.1	5.9	21	3.1	3.2	0
Harp (olig)	5.4	18	19	5.4	7.0	8	5.8	9.4	17
Minnow (eutr)	.12	42	13	12	54	17	15	35	13

(* Significant Difference - "t" test)
95% level

The increase in bacterial populations occurred within the first two weeks after introducing these compounds to the lakes. The heterotrophic populations within control sacs showed no significant change from the original concentrations at any time.

The relative increase in HPC in response to chitin and xylan was greater than the corresponding increase observed in response to starch. Hannah and Harp Lakes showed the greatest percent increase in response to added substrate above the control. Middle, Minnow and Maclean Lakes on the other hand responded less than the former oligotrophic lakes to substrates. The limed oligotrophic Middle Lake responded in a similar way to added carbon sources as the eutrophic lakes.

The acid-tolerant or aciduric bacteria (APC) increased in response to starch in all lakes. The APC increased in response to chitin and xylan in Hannah, Middle and Harp Lakes but not in Minnow Lake. The response of the aciduric population to polysaccharides was greatest in Hannah Lake, less in Middle and Harp Lakes and least in Minnow Lake.

Yeasts showed no significant response to starch or xylan in any of the lakes. With chitin as substrate, only Hannah Lake showed a significant increased yeast count over the control.

All neutral pH lakes, whether eutrophic (Minnow, Maclean) or oligotrophic (Harp, Middle) showed a significantly greater HPC geometric mean than APC geometric mean in dialysis sacs. The acidic oligotrophic Hannah Lake was the only exception where the APC responded to added polysaccharide substrate by actually exceeding the HPC.

Holm-Hansen & Booth (1966) found ATP levels in ocean water indicative of bacterial populations 50 - 2,000 times those found by plating techniques. The average bacterial cell contains about 1.5×10^{-9} µg ATP (Holm-Hansen & Booth, 1966). On this basis, the mean ATP concentration theoretically derived from the aerobic heterotrophic plate count GM was calculated for each treatment. These values were compared to the actual concentrations of ATP found in the extracted contents of the dialysis sacs. The results were presented in Table 2.2. A relatively large proportion of the actual ATP extracted was accounted for by the estimated ATP calculated from the HPC geometric means for the control treatments on Middle and Maclean Lakes and the chitin and xylan treatments on Middle and Minnow Lakes. For the dialysis sac treatments from Harp and Hannah Lakes, a significant proportion of ATP not accounted for by HPC biomass was presumably derived from organisms other than heterotrophic bacteria. For starch treatments, all lakes except Minnow had a relatively large concentration of non-heterotrophic bacterial ATP. This suggests that in Hannah and Harp Lakes, relatively more of the ATP found in dialysis sacs was due to living organisms other than heterotrophic bacteria when compared to Middle Lake and the eutrophic Minnow or Maclean Lakes.

In all lakes, dialysis sac treatments with added substrates contained a greater ATP concentration than the corresponding controls. Generally, the ATP increases paralleled the

Table 2.2 - Mean ATP concentrations in dialysis sacs incubated with and without added substrates in various lakes as compared to the estimated ATP concentration calculated from the geometric means of heterotrophic bacteria (HPC).

TREATMENT	ATP concentration (ug ml^{-1}) Mean ATP extracted from dialysis sacs	Estimated ATP associated with HPC (GM X 1.5 X 10^{-9} ug ATP)*	Estimated ATP from sources other than HPC (Percentage)
CONTROLS			
Hannah (acid cont)	4.7×10^{-4}	7.0×10^{-6}	(96%)
Middle (limed)	8.5×10^{-4}	7.8×10^{-4}	(8%)
Harp (olig)	9.8×10^{-4}	1.4×10^{-5}	(98%)
Minnow (eutr)	2.3×10^{-3}	8.3×10^{-4}	(60%)
Maclean (eutr)	1.6×10^{-4}	1.3×10^{-4}	(20%)
CHITIN			
Hannah (acid cont)	5.7×10^{-3}	9.8×10^{-4}	(80%)
Middle (limed)	9.7×10^{-3}	1.2×10^{-2}	(0%)
Harp (olig)	6.6×10^{-3}	5.0×10^{-3}	(24%)
Minnow (eutr)	1.9×10^{-2}	2.7×10^{-2}	(0%)
XYLAN			
Hannah (acid cont)	1.3×10^{-2}	9.6×10^{-3}	(27%)
Middle (limed)	1.1×10^{-2}	4.1×10^{-2}	(0%)
Harp (olig)	5.7×10^{-3}	1.2×10^{-3}	(79%)
Minnow (eutr)	2.3×10^{-2}	2.0×10^{-2}	(13%)
STARCH			
Hannah (acid cont)	2.9×10^{-3}	6.3×10^{-4}	(78%)
Middle (limed)	6.6×10^{-3}	2.3×10^{-3}	(65%)
Harp (olig)	5.7×10^{-3}	5.1×10^{-4}	(88%)
Minnow (eutr)	5.2×10^{-3}	4.6×10^{-3}	(12%)

* See text for explanation

increased counts observed for heterotrophic bacteria. A significantly greater percent increase in ATP concentration was observed in the acid oligotrophic lake compared to the other lakes.

The results of nutrient analysis for the polysaccharides employed in the dialysis sac treatments were presented in Table 2.3 . Chitin and xylan contained more nutrient contaminants than the starch. The nutrient impurities associated with xylan and chitin probably had some stimulating effect on bacterial populations, since the productivity response to starch was somewhat lower than that observed with chitin and xylan. With starch, no nutrient impurities were associated with the carbon source to give a bacterial growth response.

In principle, the cellulose nitrate dialysis sac allows free diffusion of nutrients and metabolic waste products but is impermeable to colloidal particles and microbial cells. A state of dynamic equilibrium will be reached between the concentration of soluble nutrients within the sac and the external water.

As inorganic nutrient salts are taken up by the actively growing microbial population utilizing the organic carbon sources, more of these soluble nutrient ions move into the chamber to replace those lost through uptake by active biomass. Conversely, an excess of inorganic nutrients within the sac, would diffuse out to the surrounding water. Thus nitrogen and phosphorus would not necessarily be limiting to the same extent as macromolecules.

Table 2.3 Chemical analysis of polysaccharides employed as substrates in dialysis sacs.

Nutrient Contaminants	CHITIN		XYLAN		STARCH	
	A*	B*	A	B	A	B
Protein	20	50	12	30	0.85	2.1
Soluble carbohydrates	1.7	4.3	>150	>400	>150	>400
Total inorganic phosphorus	0.14	0.36	0.17	0.41	0.02	<.04
Total inorganic N	0.12	0.31	0.08	0.23	.01	<.04

*A = mg nutrient/g substrate

*B = final nutrient concentration within dialysis sac after addition of substrate (mg/l).

The large response of aciduric bacteria in Hannah Lake to polysaccharide substrates indicated that organic carbon was limiting. Generally, bacterial populations in the oligotrophic lakes responded to carbon sources more than in the eutrophic lakes which had a naturally higher content of organic carbon. Apparently a good potential for heterotrophic bacterial production exists if available organic carbon sources are present in the lakes. Natural sources of organic carbon originating within the lake ecosystem are photosynthetic plants and algae. Exogenous sources such as soil run-off and leaf litter also contribute to organic matter in lakes. The latter sources are probably more important in oligotrophic lakes. However, a paucity of terrestrial vegetation existed around Hannah and Middle Lakes, and primary productivity of the lakes was very low. By eventually stimulating primary productivity with reclamation treatments and thus increasing the supply of organic matter in the lakes, the population of heterotrophic microorganisms would be augmented.

The heterotrophic populations, by serving as a food source directly for protozoa and rotifers, and indirectly for invertebrate grazers (Arthropoda), would assist in building up populations of these consumers and eventually fish in the complex aquatic food chain.

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- APPENDIX III -

Table 2.1-Plate count (HPC) of heterotrophic bacteria found in dialysis sac contents at various incubation times *in situ* in various lakes, summer, 1974.

	HPC ml^{-1} TIME (WKS) 0	2	4	6	8	10
CHITIN						
Hh*	1.3×10^3	1.8×10^6	6.6×10^6	9.1×10^5	8.5×10^4	3.9×10^5
Me	6.3×10^4	4.2×10^7	7.3×10^6	9.4×10^6	-	5.0×10^6
Hp	2.2×10^3	1.7×10^6	6.5×10^5	-	8.2×10^6	1.6×10^7
Mw	3.1×10^5	3.6×10^7	1.0×10^7	-	-	-
Mcn	8.6×10^4	1.45×10^7	4.3×10^7	6.8×10^6	-	-
XYLAN						
Hh	3.3×10^2	1.5×10^6	7.4×10^6	-	1.8×10^6	6.7×10^5
Me	2.2×10^4	3.1×10^7	5.1×10^7	-	9.6×10^6	8.4×10^6
Hp	8.2×10^2	1.9×10^6	3.6×10^5	-	7.5×10^5	-
Mw	6.5×10^4	5.9×10^7	1.3×10^7	6.4×10^6	-	-
Mcn	1.4×10^5	6.9×10^7	3.9×10^6	1.6×10^7	-	-
STARCH						
Hh	5.3×10^2	6.8×10^5	7.7×10^5	8.2×10^5	-	3.2×10^5
Me	4.0×10^4	5.7×10^6	-	2.4×10^6	8.3×10^5	-
Hp	7.8×10^2	8.8×10^4	-	6.9×10^5	-	8.0×10^5
Mw	6.9×10^4	5.5×10^6	4.8×10^6	9.7×10^5	-	-
CONTROLS						
Hh	6.7×10^2	6.0×10^3	8.7×10^3	8.5×10^2	3.1×10^2	-
Me	7.5×10^4	6.2×10^5	4.3×10^5	2.5×10^4	-	-
Hp	7.3×10^3	2.3×10^4	5.9×10^3	-	5.7×10^4	-
Mw	2.5×10^5	7.6×10^5	1.2×10^6	3.4×10^6	-	-
Mcn	8.0×10^4	3.0×10^5	4.8×10^5	1.3×10^5	-	-

* Hh = Hannah
Me = Middle
Hp = Harp
Mw = Minnow
Mcn = Maclean

Table 2.2- Acid tolerant heterotrophic bacterial counts found in dialysis sacs taken from lakes at various times during summer, 1974.

	ACIDURIC COUNT ml^{-1} TIME (WKS)					
	0	2	4	6	8	10
CHITIN						
Hh *	6.1×10^3	6.2×10^5	5.3×10^6	-	6.0×10^4	-
Me	6.8×10^2	9.2×10^3	7.2×10^3	-	7.3×10^4	-
Hp	8.2×10^2	4.1×10^5	-	1.2×10^5	3.8×10^4	-
Mw	5.3×10^2	1.1×10^5	8.5×10^4	2.7×10^4	-	-
XYLAN						
Hh	1.7×10^3	8.6×10^5	6.4×10^6	-	5.7×10^6	-
Me	2.8×10^2	1.8×10^5	6.2×10^5	-	4.7×10^4	-
Hp	3.0×10^3	8.2×10^4	3.5×10^5	-	6.1×10^4	-
Mw	4.4×10^3	9.0×10^4	5.2×10^5	4.3×10^4	-	-
STARCH						
Hh	9.2×10^2	6.2×10^5	-	4.4×10^5	2.0×10^5	-
Me	1.7×10^3	6.8×10^4	2.0×10^4	-	2.3×10^3	-
Hp	6.1×10^2	4.3×10^4	1.1×10^4	-	6.0×10^4	-
Mw	2.3×10^2	4.8×10^4	3.8×10^4	2.0×10^4	-	-
CONTROLS						
Hh	8.8×10^2	1.1×10^3	8.6×10^2	5.5×10^1	4.7×10^1	-
Me	4.8×10^2	3.7×10^3	5.3×10^2	4.5×10^2	-	-
Hp	2.4×10^2	1.3×10^3	-	3.6×10^3	8.1×10^3	-
Mw	6.2×10^2	3.6×10^3	9.4×10^2	7.0×10^3	-	-

* See Appendix III , Table 2.1

Table 2.3-ATP concentration found in dialysis sac contents extracted from lakes at various time intervals.

LAKE & TIME	(μg ATP ml^{-1}) CHITIN	XYLAN	STARCH	CONTROL I	CONTROL II
HANNAH					
4 wks	1.00×10^{-2}	1.31×10^{-2}	3.60×10^{-3}	6.22×10^{-4}	4.8×10^{-4}
8 wks	3.80×10^{-3}	8.25×10^{-3}	2.70×10^{-3}	3.30×10^{-4}	1.4×10^{-4}
10 wks	3.18×10^{-3}	1.85×10^{-2}	2.28×10^{-3}	-	-
MIDDLE					
4 wks	1.32×10^{-2}	8.03×10^{-3}	5.51×10^{-3}	8.51×10^{-4}	6.58×10^{-4}
8 wks	6.38×10^{-3}	1.13×10^{-2}	6.59×10^{-3}	-	4.28×10^{-4}
10 wks	9.51×10^{-3}	1.47×10^{-2}	7.55×10^{-3}	-	-
HARP					
4 wks	8.44×10^{-4}	2.50×10^{-3}	2.83×10^{-3}	4.24×10^{-4}	7.25×10^{-4}
8 wks	9.41×10^{-3}	6.88×10^{-3}	5.86×10^{-3}	1.43×10^{-3}	-
10 wks	1.91×10^{-2}	7.78×10^{-3}	8.49×10^{-3}	-	1.52×10^{-4}
MINNOW					
4 wks	1.35×10^{-2}	2.34×10^{-2}	6.97×10^{-3}	2.21×10^{-3}	2.47×10^{-3}
8 wks	-	-	3.49×10^{-3}	-	2.19×10^{-3}

Fig. 2.1-Productivity of heterotrophic bacterial populations in dialysis sacs incubated in various lakes using chitin as substrate during late summer, 1974.

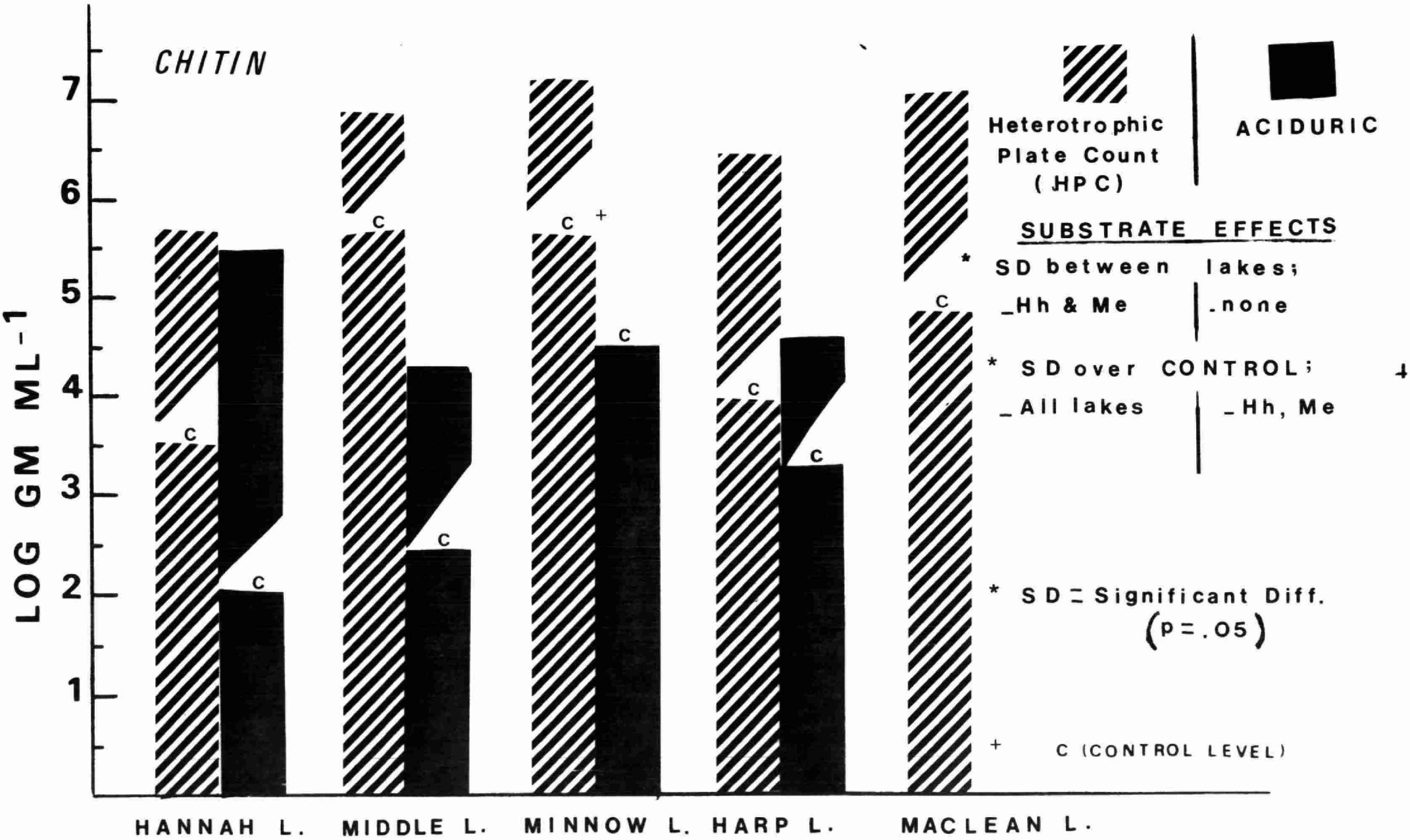

Fig. 2.2 - Productivity of heterotrophic bacterial populations in dialysis sacs incubated in various lakes using hemicellulose (xylan) as substrate.

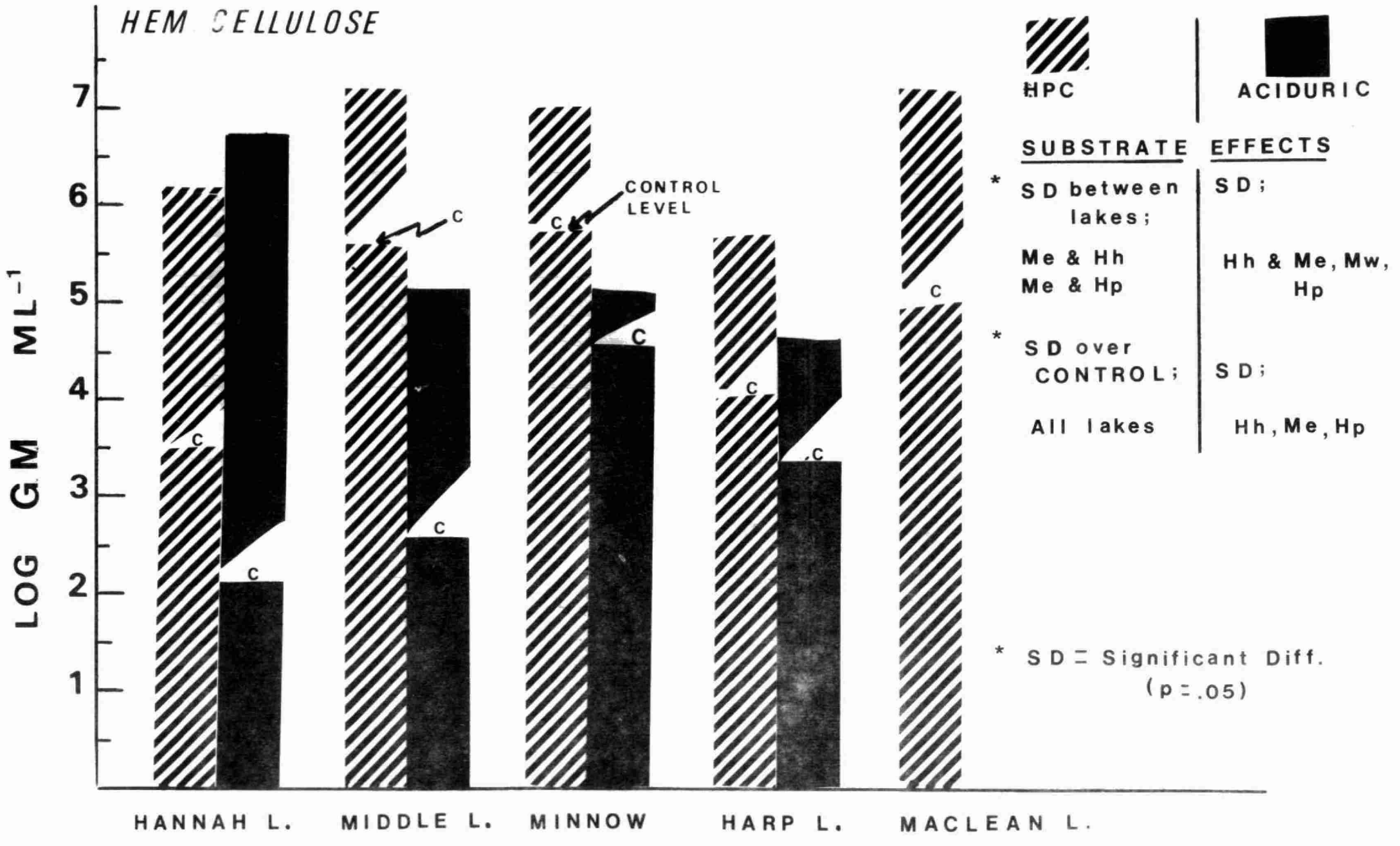

Fig. 2.3 - Productivity of heterotrophic bacterial populations in dialysis sacs incubated in various lakes using starch as substrate.

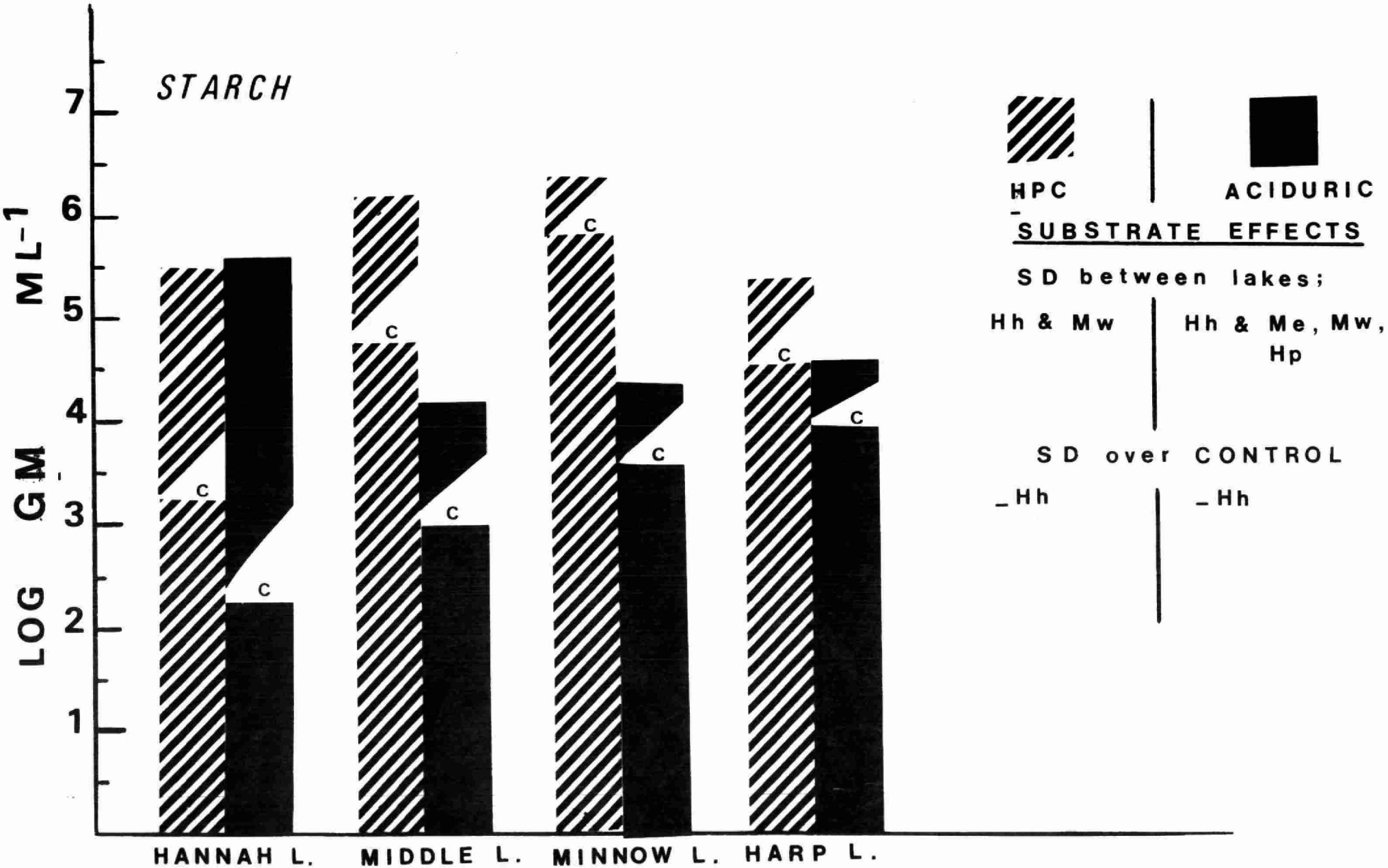

Fig. 2.4 - Response of yeast populations to chitin and hemicellulose in dialysis sacs incubated in various lakes during late summer, 1974.

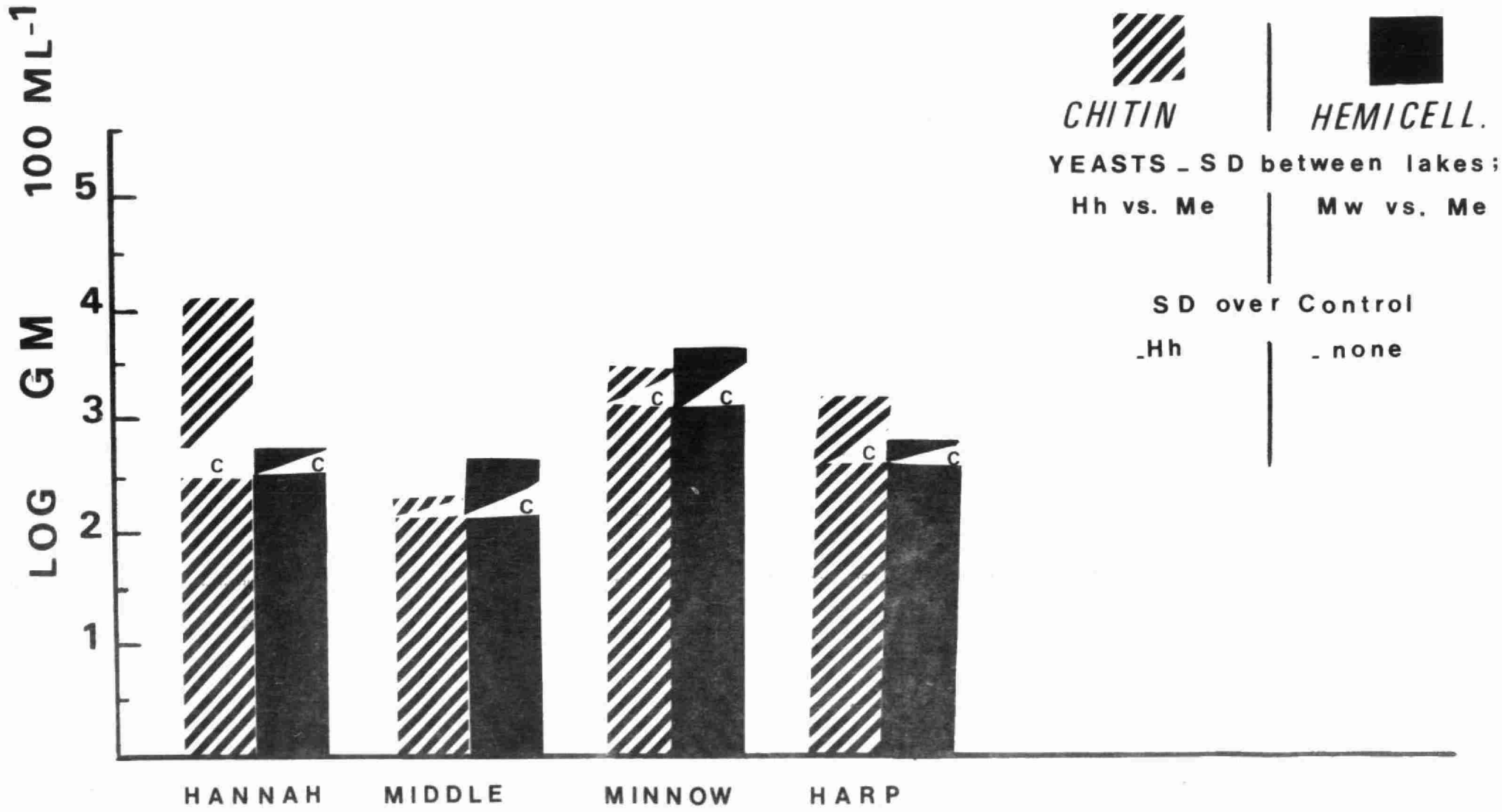

Fig. 2.5 - Biomass production as measured by ATP concentration in dialysis sacs incubated with and without carbon substrate additions in various lakes during late summer, 1974.